Stable isotopes provide independent support for the use of mesowear variables for inferring diets in African antelopes

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Summary

We examine the relationship between mesowear variables and carbon and nitrogen isotopes in 16 species of African antelope (Mammalia: Bovidae). We show significant differences in carbon and nitrogen isotope values between individuals exhibiting sharp versus round cusps, and high versus low occlusal relief. We show significant correlations between mesowear variables and both carbon and nitrogen isotopes. We find significant correlations between mesowear score (MS) and nitrogen, but not carbon isotopes. Finally, we find no significant correlations between hypsodonty index and either isotope examined. Our results provide strong support for the use of mesowear variables in palaeodietary reconstructions of antelopes. Our results further suggest that for the antelopes examined here, mesowear signals are a direct result of diet while hyposodonty may be the result of phylogenetic legacy.

Key words: mesowear, palaeodiet, stable isotope, nitrogen, carbon

1. Introduction

Mesowear – the macroscopic wear observed on teeth – is a now common means of inferring the dietary preferences of modern and extinct mammals (e.g. [1-3]). It relies on the hypothesis that the appearance of wear facets on the molar teeth of herbivores will correlate with the level of abrasiveness in a species' diet. Two types of tooth wear – attrition and abrasion – have been observed to affect teeth in quantifiably different ways [4, 5]. Attrition refers to the formation of wear facets through tooth-on-tooth contact, and is thought to lead to sharper cusps and higher occlusal relief. This type of wear is most common in browsers (defined as species that include at least 70% dicots in their diet [6]). Abrasive wear occurs through tooth-on-food contact and generally creates more rounded cusps and lower occlusal relief due to the dietary presence of intrinsic abrasive particles such as phytoliths or forage qualities such as toughness or nutritive value [5]. Hence, original mesowear analyses examined the relative proportion of two variables – sharpness of cusps and occlusal relief – summarised in a categorical manner across a wide range of species (figure 1). The use of these variables allows fossil species of unknown diet to be classified to one of several broad dietary categories [4]. Later refinement of the mesowear technique led to the conversion of the mesowear signal into a single, continuous variable, referred to as the mesowear score (MS) [3, 7]. Components of the mesowear score have been correlated with biotic and abiotic variables including precipitation, hypsodonty index and microwear as well as broad categorical dietary classes (e.g. [5, 8]).

Another way of inferring diets is through stable isotope analysis. Carbon and nitrogen isotopes found in an animal's body tissue, including bones, teeth and hair are derived from dietary carbon and nitrogen [9] and in the case of hair particularly reflect the protein component of the diet [10]. Carbon isotopes in body tissues of herbivores reflect the proportion of C3/C4 plants in their diet. C3 plants (largely browse) have a δ^{13}C values ranging from -32‰ in closed understory canopy conditions to -21‰ in more open environments [11], while C4 plants (including many tropical grasses) can range from -21‰ to -9‰. Nitrogen isotopes will vary with trophic level, although other variables such as soil, climatic and inter- and intraspecific physiological differences can produce significant shifts in δ^{15}N values [9]. Isotopes found in mammal hair preserve the record of the diet spanning months to years [12]. Similarly mesowear is reported to record dental wear over the last few months or years of an animal's life. Therefore a significant correlation between isotopes found in hair and mesowear variables should be expected. However, this relationship has never before been tested. Here, we test such a correlation by comparing mesowear variables with carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotopes in 16 species of African antelopes (Mammalia: Bovidae).

2. Materials and Methods

A total of 104 wild-caught antelope specimens representing 16 species from the Koninklijk Museum voor Midden-Afrika (Tervuren, Belgium) were sampled for both mesowear variables and carbon and nitrogen stable isotopic composition.

Linear measurements (ORL occlusal length; ORH occlusal height; CH crown height, BLW bucco-lingual width) were taken using digital callipers from both upper and lower molars (figure 2(a)). These measurements were considered because they enable both occlusal relief and hypsodonty index to be quantified. Mesowear variables for the upper second molars of each specimen were measured per Louys et al. [13]: sharpness was determined by examining the sharpest cusp and scored as sharp, round or blunt, while occlusal relief was determined by dividing ORH by ORL, although in cases where the cusps were chipped this was determined visually. Hypsodonty index (HI) was calculated by dividing CH by BLW for the lower third molar. For each specimen yielding mesowear scores, at least 1g of hair was extracted from the pelt of the same specimen. Hair samples were cleaned by wiping with acetone to remove any adhering dirt and grease as described by Cerling et al. [14]. Sub-samples of the clean hair were weighed into tin capsules and analysed for their nitrogen and carbon stable isotopic composition by continuous flow isotope ratio mass spectrometry using a Sercon 20/22 IRMS coupled to a Sercon GSL sample combustion device using Helium carrier gas. Sample data are reported in standard delta per mil notation (δ ‰) relative to V-PDB and AIR international standards for carbon and nitrogen respectively.

Mann-Whitney U-tests were used to determine whether specimens having high or low occlusal relief showed significant differences in $\delta^{13}C$ and $\delta^{15}N$ values,

while the Kruskal-Wallis test with post-hoc Mann-Whitney was used to assess these differences in specimens having sharp, round or blunt cusps. Correlation between raw molar measurements, mesowear score or hypsodonty index and $\delta^{13}C$ and $\delta^{15}N$ was assessed using Kendall's τ, and significance was assessed at $\alpha = 0.05$. The correlation between $\delta^{13}C$ and $\delta^{15}N$ values and sharpness (scored as 0 = sharp, 1 = round, 2 = blunt), occlusal relief (0 = high, 1 = low) and mesowear score (0 = high relief and sharp cusps, 1 = high relief and round cusps, 2 = low relief and sharp cusps, 3 = low relief and round cusps, 4 = low relief and blunt cusps) was assessed by both Kendall's τ and polyserial correlation, with significance assessed at $\alpha = 0.05$ (it is noted that the $\delta^{13}C$ are not normally distributed and hence the results from the polyserial analysis should be viewed with caution).

For species with a sample size ≥ 5, average mesowear score and the proportion of specimens having sharp, round and blunt cusps and high occlusal relief was calculated. The correlation between these and average $\delta^{13}C$ and $\delta^{15}N$ was again assessed using Kendall's τ at $\alpha = 0.05$. All analyses run on PAST ver. 2.14 [15], with any broken or unattainable measurements scored as missing values.

3. Results

Tables containing the tooth measurements and mesowear measurement results as well as the stable isotopic results are given in the supplementary data for this paper.

Both $\delta^{13}C$ and $\delta^{15}N$ show significant correlations with almost all molar metrics across all teeth examined (Table 1). However no correlation is found between crown height and $\delta^{13}C$ in all teeth save the lower third molar, and in all first molars there is no significant correlation between occlusal breadth and $\delta^{13}C$. The upper second molar has the highest average correlation with $\delta^{13}C$ in all measurements. Our findings therefore support the use of this tooth preferentially for mesowear analyses (per [4]), and forms the basis of all other analyses conducted herein.

A Kruskal-Wallis test indicates that there are significant differences between $\delta^{13}C$ values in antelopes exhibiting different cusp sharpness ($H = 7.465$, $p = 0.02393$). Mann-Whitney pairwise comparisons indicate that the only significant differences are between antelopes with sharp and rounded cusps ($p = 0.01084$), with no significant differences observed between blunt cusps and either sharp ($p = 0.8673$) or round ($p = 0.215$) cusps. No significant differences occur between $\delta^{15}N$ values in antelopes exhibiting different cusp sharpness ($H = 3.534$, $p = 0.1708$). Significant differences between $\delta^{13}C$ and $\delta^{15}N$ values in antelopes exhibiting different occlusal heights is observed ($p = 0.0449$ and $p < 0.001$, respectively).

Our analyses show no significant correlation between HI and either carbon or nitrogen isotopes (figure 2(b)). We do find a significant correlation between MS and HI (figure 2(c)) and $\delta^{15}N$ (figure 2(d), solid line), but not $\delta^{13}C$ (figure 2(d), dotted line). There were significant correlations between $\delta^{13}C$ and both cusp

sharpness and occlusal height (figure 3(a), 3(b), respectively). While a significant correlation was also found between δ^{15}N and occlusal height (figure 3(d)), no such result was observed with cusp sharpness (figure 3(c)).

Only 10 of the 16 species examined had sufficient specimens to be included in our species-averaged analysis: the bay duiker (*Cephalopus dorsalis*), the white-bellied duiker (*C. leucogaster*), the black-fronted duiker (*C. nigrifrons*), the yellow-backed duiker (*C. silvicultor*), the Weyn's duiker (*C. weynsi*), the waterbuck (*Kobus ellipsiprymnus*), the blue duiker (*P. monticola*), the reedbuck (*Redunca redunca*), the common duiker (*Sylvicapra grimmia*) and the bushbuck (*Tragelaphus scriptus*). Our species-averaged analysis is summarized in Table 2. The only significant correlation at $\alpha = 0.05$ is between the proportion of rounded cusps and average δ^{13}C.

4. Discussion

To our knowledge, this is the first study examining correlations between mesowear variables, HI and stable isotopes in any extant taxa. Our analyses show that traditional mesowear variables are highly informative about antelope diet. In particular, there are significant relationships between δ^{13}C and both sharpness of cusps and occlusal relief. The positive correlation between sharpness and δ^{13}C is consistent with the underlying rationale for mesowear analyses: as the amount of C4 (and hence tropical grass) in the diet increased, cusps decreased in sharpness. An opposite and more unexpected pattern was found in occlusal relief – as the amount of C4 decreased, low relief increased.

This may be due to the high number of frugivorous species in our analysis, whose mesowear patterns can act more like those of grazers than browsers [13].

There were significant differences between specimens exhibiting high and low occlusal reliefs for both $\delta^{13}C$ and $\delta^{15}N$. Significant differences in $\delta^{13}C$ are also found between specimens exhibiting sharp versus round cusps. That no significant differences were found between blunt cusped specimens and other specimens confirms that blunt cusps are not diagnostic for distinguishing antelope diets [13]. Mesowear score is also found to correlate significantly with $\delta^{15}N$, although not with $\delta^{13}C$, which may be a result of the high number of frugivorous species examined.

Surprisingly, compared to the specimen-by-specimen analysis the species-averaged analyses did not correlate with mesowear variables as robustly. Finding both undamaged second molars and associated pelts for a great number of specimens per species proved difficult, and these results most likely reflect the decreased sample sizes in the specimen-based analyses. However, they do indicate that a specimen-by-specimen approach may more accurately predict diet than a broad species-averaged approach. Our results suggest that in future, mesowear analyses could be used on individual specimens using an ecomorphological taxon-free approach. This study has implications for understanding the relationship between diet and dental wear. Interestingly, we found very little correlation between HI and either $\delta^{15}N$ or $\delta^{13}C$. This suggests that the abundance of C4 foods in a mammal's diet may bear no relation to their

hypsodonty index, and could explain why Pleistocene brachydont camelids from Bolivia exhibit isotopic C4 diets [16]. Codron et al. [17] found that morphological differences in antelope craniodental adaptations did not reflect varying proportions of C3 browse to C4 grass consumed, although they did reflect changes in δ^{15}N. Our study supports these findings for δ^{15}N in a related, though different set of antelopes and dental measurements. Unlike Codron et al. we did find significant correlations between δ^{13}C and most dental metrics; however these disappeared when examining the combined mesowear score, and we found no correlations at all between HI and isotopes. Similarly, Raia et al. [18] suggested that the artiodactyl mandible shape was a strongly influenced by hyposodonty, which, for our subset of antelopes at least, we demonstrate is correlated with neither δ^{13}C nor δ^{15}N. Raia et al. [18] further demonstrate that hypsodonty in artiodactyls has a very strong phylogenetic signal. Hence, for the antelopes examined here, hypsodonty may be driven more by phylogenetic legacy whereas mesowear directly reflects wear differences resulting from C3/C4 diets.

Acknowledgments

We thank E. Gilissen and W. Wendelen (RMCA) for their generous assistance in collecting the data. Financial support for this project was obtained from the Leverhulme Trust (F/00 754/C) and Synthesys grant BE-TAF-4886.

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Figure 1. Stylised antelope teeth showing typical mesowear variables.

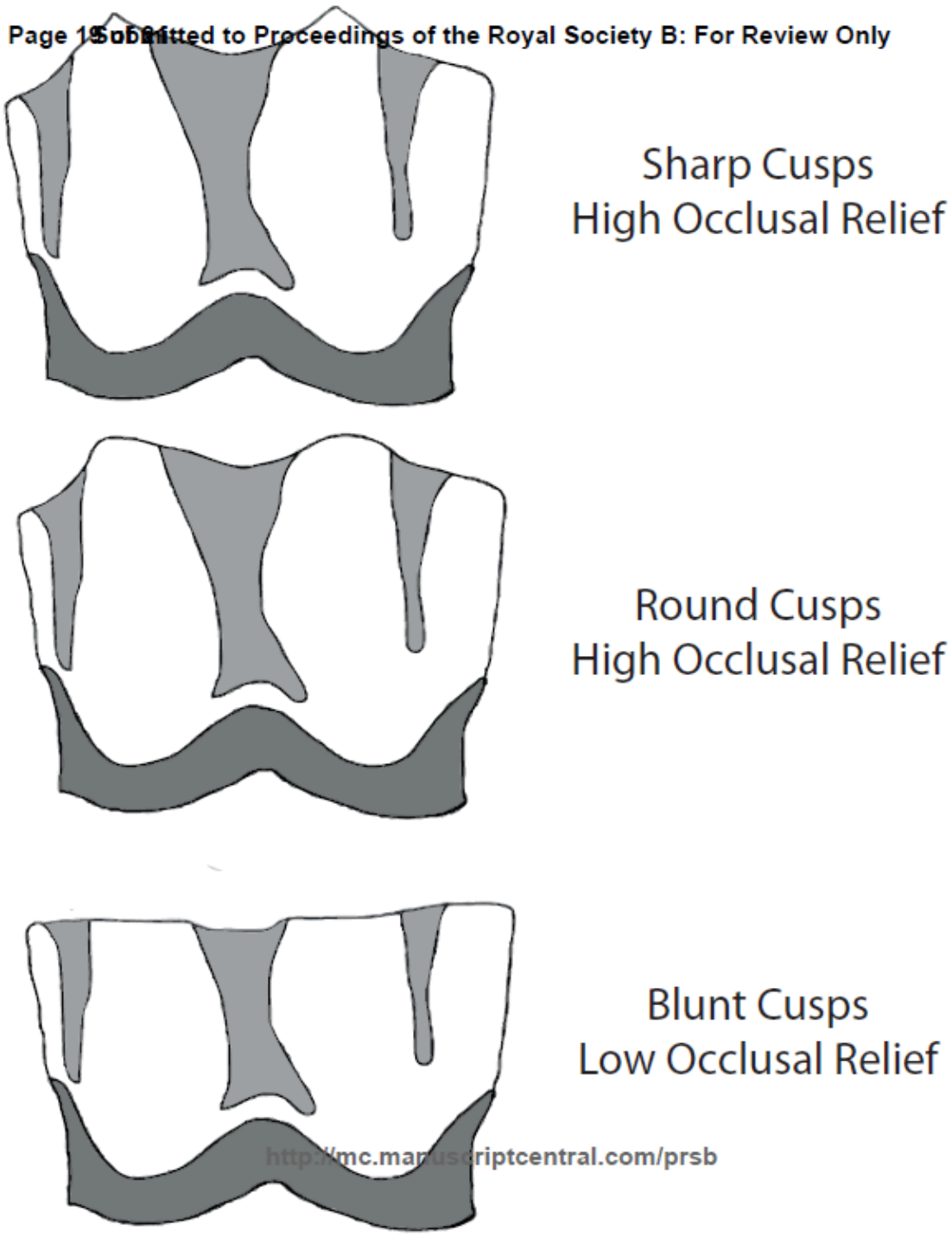


Figure 2. Measurements and correlations for hypsodonty index (HI) and mesowear score (MS). (a) stylised antelope tooth and measurements taken: ORL occlusal length; ORH occlusal height; CH crown height, BLW bucco-lingual width; (b) correlation between stable isotopes and HI: dotted line and circles represents $\delta^{13}C$; solid line and squares $\delta^{15}N$; (c) correlation between HI and MS; (d) correlation between stable isotopes and MS: dotted line and circles represents $\delta^{13}C$; solid line and squares $\delta^{15}N$; C, correlation between HI and MS

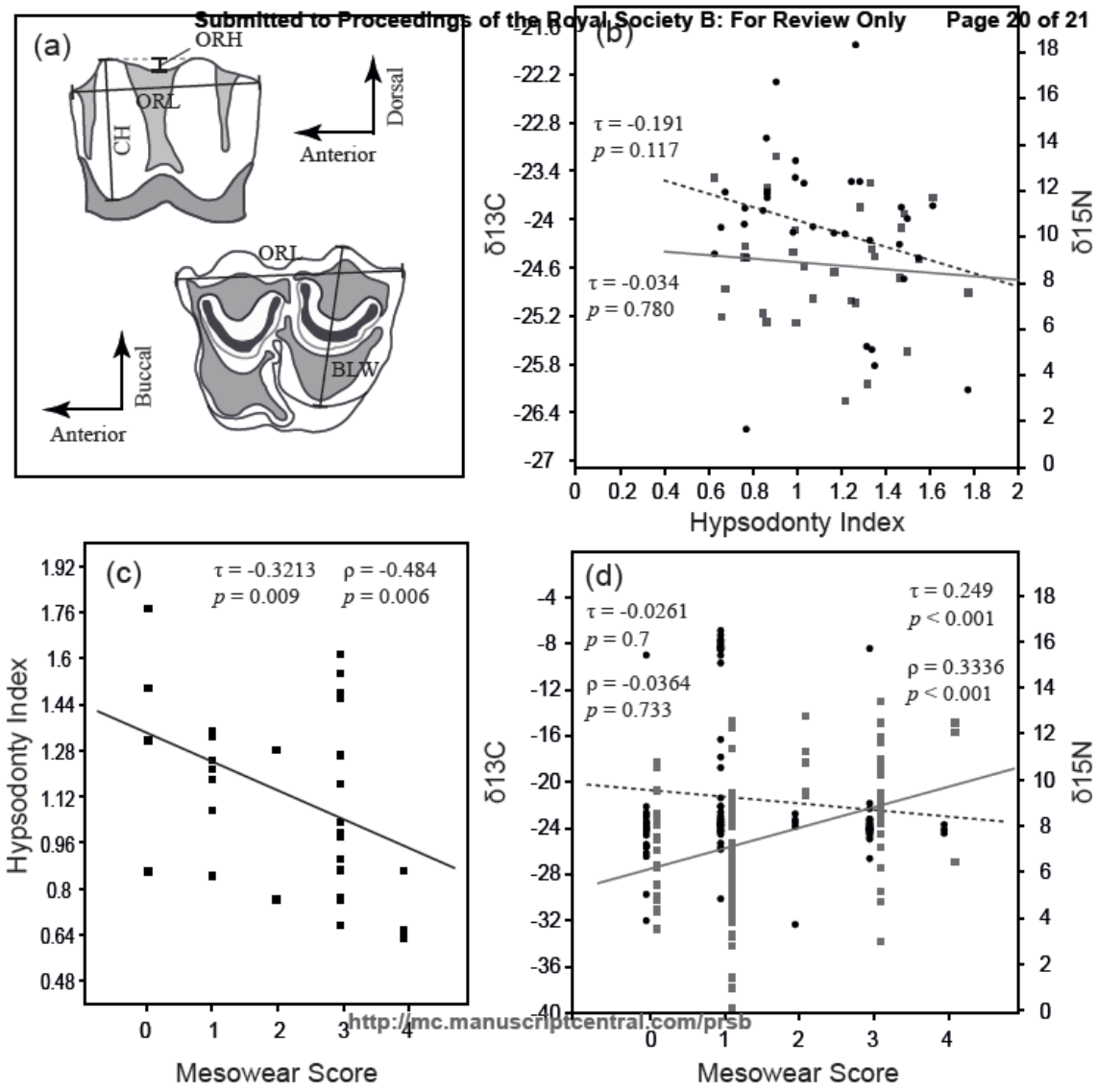
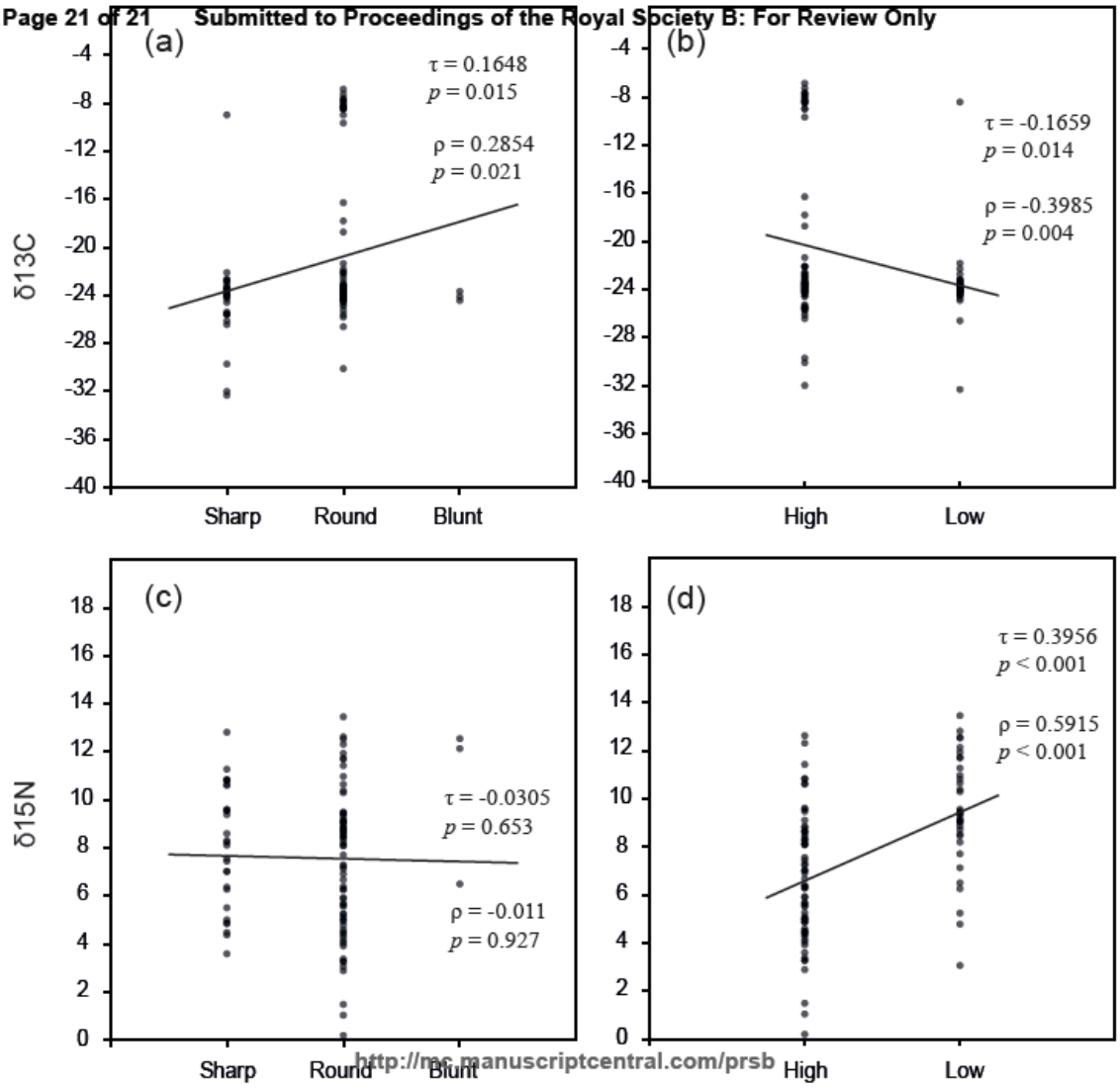


Figure 3. Correlation between mesowear variables and isotopes. (a) $\delta^{13}C$ and sharpness; (b) $\delta^{13}C$ and occlusal relief; (c) $\delta^{15}N$ and sharpness; (d) $\delta^{15}N$ and occlusal relief



Tooth	Isotope		ORL	ORH	CH	BLW
M1	δ^{13}**C**	Kendall's T	0.22534	0.194	**0.053735**	0.13781
		p(uncorr)	0.000744	0.014474	**0.46383**	0.042196
	δ^{15}**N**	Kendall's T	-0.28498	-0.35226	-0.19718	-0.18947
		p(uncorr)	1.99E-05	8.99E-06	0.007187	0.005222
M2	δ^{13}**C**	Kendall's T	0.22076	0.221	**0.039181**	**0.059504**
		p(uncorr)	0.000952	0.002296	**0.6366**	**0.3755**
	δ^{15}**N**	Kendall's T	-0.28999	-0.41152	-0.22632	-0.14029
		p(uncorr)	1.42E-05	1.37E-08	0.006351	0.036673
M3	δ^{13}**C**	Kendall's T	0.16858	0.11695	**-0.09266**	**0.040692**
		p(uncorr)	0.012049	0.089678	**0.39972**	**0.54859**
	δ^{15}**N**	Kendall's	-0.31339	-0.38522	-0.3297	-0.18428

		T				
		p(uncorr)	3.05E-06	2.27E-08	0.002733	0.006595
m1	δ^{13}**C**	Kendall's T	**0.13439**	0.26321	**0.031797**	0.15428
		p(uncorr)	**0.056375**	0.002723	**0.67044**	0.028493
	δ^{15}**N**	Kendall's T	-0.30577	-0.39604	-0.20024	-0.25784
		p(uncorr)	1.42E-05	6.48E-06	0.007368	0.000251
m2	δ^{13}**C**	Kendall's T	0.17139	0.18058	**-0.10071**	**0.079732**
		p(uncorr)	0.014959	0.017025	**0.25142**	**0.26029**
	δ^{15}**N**	Kendall's T	-0.3039	-0.35932	-0.1509	-0.17038
		p(uncorr)	1.60E-05	2.05E-06	0.085712	0.016152
m3	δ^{13}**C**	Kendall's T	**0.12206**	0.15074	-0.36673	**0.00307**
		p(uncorr)	**0.086597**	0.045004	0.002289	**0.96602**

	δ^{15}**N**	Kendall's T	-0.27374	-0.42418	-0.25691	-0.19379
		p(uncorr)	0.000122	1.69E-08	0.032629	0.00716

Table 1. Correlation between individual measurements and carbon and nitrogen isotope values assessed using Kendall's τ, uncorrelated variables indicated in bold. Abbreviations: ORL occlusal length; ORH occlusal height; CH crown height, BLW bucco-lingual width

		%Sharp	%Round	%Blunt	%High	Average MS
Average δ^{13}C	Kendall's T	-0.2046	**0.49441**	-0.42601	0.23002	-0.022222
	p(uncorr)	0.41023	**0.046594**	0.086411	0.35454	0.92873
Average δ^{15}N	Kendall's T	0.022733	-0.22473	0.48686	-0.36803	0.46667
	p(uncorr)	0.9271	0.36571	0.050044	0.13852	0.060341

Table 2. Correlation between species-averaged mesowear variables and carbon and nitrogen isotope values assessed using Kendall's τ, correlated variables indicated in bold. Abbreviations: MS mesowear score